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Neochrosporium camelliae-sinensis gen. & sp. nov. from tea in China

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Abstract —A new hyphomycete genus and species, *Neochrosporium camelliae-sinensis*, was isolated from the leaves of Xinyang Maojian tea. Morphological and molecular (ITS, LSU) analyses indicated that the strain represented a new genus *Neochrosporium* with the type of *Neochrosporium camelliae-sinensis*. The distinct morphological features are as follows: white PDA colonies, 0-septate phialidic conidia, and absence of ramoconidia.

Key words —phylogenetic analysis, hyphomycete, *Pleosporales*, *Theaceae*

Introduction

Tea is a beverage made from *Camellia sinensis* (L.) Kuntze (*Theaceae*). Tea plants are hardy evergreen shrubs or small trees and are one of the most important economic crops in China. Tea drinking has a long history in China. In recent years, there have been a number of studies on tea plant microorganisms, the microbial species contained in them are important for the study of tea plant microorganisms as well as microbial control (Lu & al. 2007).

Cladosporium-like hyphomycetes are widespread in the world, including endophytic, phytopathogenic, and saprobic species (Crous & al. 2007). The ITS and LSU sequences have been used to re-evaluate *Cladosporium*-like taxa (Braun & al. 2003; Crous & al. 2007). *Ochrocladosporium* is one genus of *Cladosporium*-like fungi with ramoconidia (Pereira & al. 2022).

Ochrocladosporium was erected by Crous & al. (2007) with the type species *O. elatum* (Harz) Crous & U. Braun and is characterized by mycelium composed of branching septate hyphae, conidia solitary, erect, subelliptical. Colonies on PDA are rich in hyphae with a large number of production bags and uniform edges, ramoconidia present, and conidia occurring in branched chains (Crous & al. 2007). In this study, a new endophytic strain was obtained from fresh tea leaf, with sequences of ITS and LSU very similar to *Ochrocladosporium elatum*, but ramoconidia absent. The strain is proposed here as *Neochrosporium camelliae-sinensis*, in a new monotypic genus.

Materials and methods

Isolation

Tea tissue samples in polyethylene bags (Singh & Kamal 2012) were removed and washed with sterile water. They were cultured on PDA medium by tissue isolation method to isolate and purify the fungus in an incubator at 25°C for 7–15 days. Fungi were mounted in a drop of lactophenol oil on microscope slides. Species identification was performed based on morphological characteristics of the fungi (Fu & al. 2019) using a Nikon N2 optical microscope (Nikon Y-IDT, JAPAN). The holotype and ex-type culture are deposited in the Fungal Herbarium, Henan Agricultural University, Zhengzhou, China (HHAUFF).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fresh mycelium using CTAB method (Doyle 1991). Primers ITS1 and ITS4 (White & al. 1990) were used to amplify the rDNA ITS region; LR5 and LROR (Stielow & al. 2015) were used for LSU region (Fernández & al. 1999). The PCR mixture was amplified using an Eppendorf MasterCycle gradient thermal cycler (Eppendorf, Hamburg, Germany) with the procedure: initial denaturation at 94°C for 5 min; 35 cycles of 45 s at 94°C, 30 s at 55°C, and 90 s at 72°C; final extension at 72°C for 10 min, and incubation at 4°C. PCR products were detected on a 1% agarose gel, and sequences were obtained on an ABI 3730XL automated DNA Analyzer at Sangon Biotech (Shanghai) Co. Ltd.

Sequence alignment and phylogenetic analyses

The bidirectional sequences obtained from LSU were spliced by using DNAMAN software (Liu & al. 2011), and the spliced sequences were assembled with the sequences from ITS. The sequences were blasted in GenBank (Sayers & al. 2022), and related sequences were downloaded and included in the analysis (Table 1). The sequences were manually aligned and edited using MEGA7 (Kumar & al. 2016) and the trees were built by MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003) with 10 million generations bootstrap. The run was ended when the mean standard deviation of the split frequency was below 0.01.

Table 1. Species and sequences used in the phylogenetic analyses. New sequences are in bold.

Family	Species name	Strain	GenBank no.	
			ITS	LSU
Stachybotryaceae	*Alfaria acaciae*	CPC 31940	MH107882	MH107930
Pleosporales, incert.	*Camarosporidiella elaeagnicola*	MFLU15-1924	MF434135	MF434223
Didymellaceae	*Cladosporium bruhnei*	CBS 723.79	EU167558	KC800751
Didymellaceae	*Cladosporium* sp.	AIT_F1186	MF276663	MF276663
Leptosphaeriaceae	*Coniothyrium wernsdorffiae*	CBS 150.34	MH855474	MH855474
Dothioraceae	*Dothiora pyrenophora*	CPC 30634	KY929146	KY929179
Pleosporales, incert.	*Foliophoma fallens*	CBS 284.70	KY929148	GU238078
Pleosporales, incert.	***Neochrosporium camelliae-sinensis***	**HHAUF220225**	**OP605503**	**OP623515**
Cucurbitariaceae	*Neocucurbitaria rhamni*	C133	MF795777	MF795777
Cucurbitariaceae	*Neocucurbitaria rhamni*	C190	MF795778	MF795778
Cucurbitariaceae	*Neocucurbitaria rhamni*	C277	MF795779	MF795779
Pleosporales, incert.	*Ochrocladosporium elatum*	CBS 146.33	EU040233	EU040233
Pleosporales, incer.	*Ochrocladosporium frigidarii*	CBS 103.81	EU040234	EU040234
Pleosporales, incert.	*Parapleurotheciopsis inaequiseptata*	MUCL 41089	EU040235	EU040235
Thyridariaceae	*Parathyridaria philadelphi*	CBS 143432	MH107905	NG059873
Thyridariaceae	*Parathyridaria robiniae*	MFLUCC 14-1119	NR168161	KY511141
Phaeosphaeriaceae	*Phaeosphaeria avenaria*	AFTOL-ID 280	AY544725	AY544684
Cladosporiaceae	*Rachicladosporium luculiae*	CPC 11407	EU040237	EU040237
Didymellaceae	*Stagonosporopsis caricae*	HEYB4	MZ145054	MZ145055

Phylogenetic results

The final data set included information from 18 strains. The phylogenetic tree (Fig. 1) indicates that our *Neochrosporium* strain clustered with the two *Ochrocladosporium* strains (*O. elatum* and *O. frigidarii*); and this clade was sister group of *Necucurbitaria* and *Parathyridaria* strains.

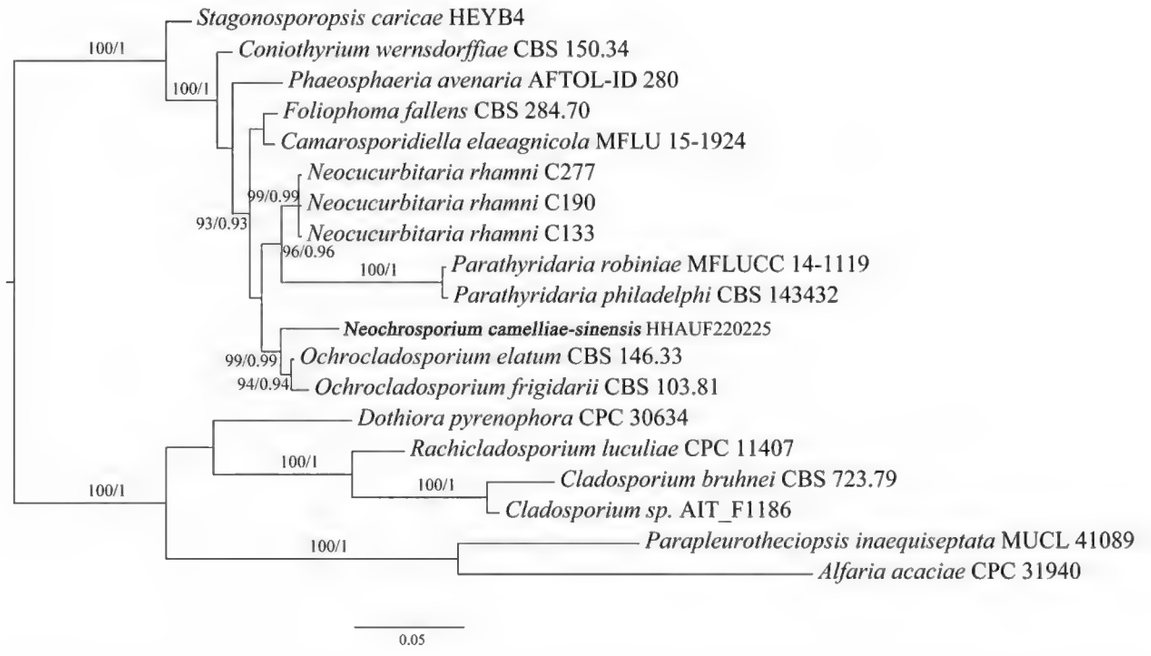

Fig. 1. Phylogenetic tree for *Neochrosporium camelliae-sinensis* and similar *Cladosporium*-like species, inferred from combined ITS and LSU sequence alignment. Branches are labeled with maximum-likelihood bootstrap support ≥90% and Bayesian posterior probability ≥0.9. New sequence indicated in bold.

Taxonomy

Neochrosporium R.P. Liu, Meng Zhang & Y.H. Geng, **gen. nov.**

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Differs from *Ochrocladosporium* by its white PDA colonies, its 0-septate phialidic conidia, and its lack of ramoconidia.

Type species: *Neochrosporium camelliae-sinensis* R.P. Liu & al.

Etymology: related to the genus of *Ochrocladosporium*.

Mycelium consisting of brached, smooth, hyaline hyphae. Differs from *Ochrocladosporium* by its white colonies in PDA, its 0-septate phialidic conidia, and its lack of ramoconidia. Colonies in shades of brown; the back of the colonies were slightly white. Conidiophores micronematous. Conidiogenous cells monophialidic integrated, subcylindrical to doliiform, pale brown, smooth; determinate to sympodial, loci conically truncate, subdenticulate. Conidia 0-septate, oval, hyaline to pale brown, thin-walled, smooth to verruculose. Teleomorph unkown.

Neochrosporium camelliae-sinensis R.P. Liu, Meng Zhang & Y.H. Geng, **sp. nov.** FIG. 2

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Differs from all related *Cladosporium*-like hyphomycetes by its white PDA colonies, and its lack of ramoconidia.

TYPE: China, Henan Province, Xinyang, *Camellia sinensis* leaf, 26 September 2021, R.P. Liu (**Holotype**, HHAUF220225; ex-type culture, HHAUF220225; GenBank OP605503, OP623515)

ETYMOLOGY: named after the host *Camellia sinensis*.

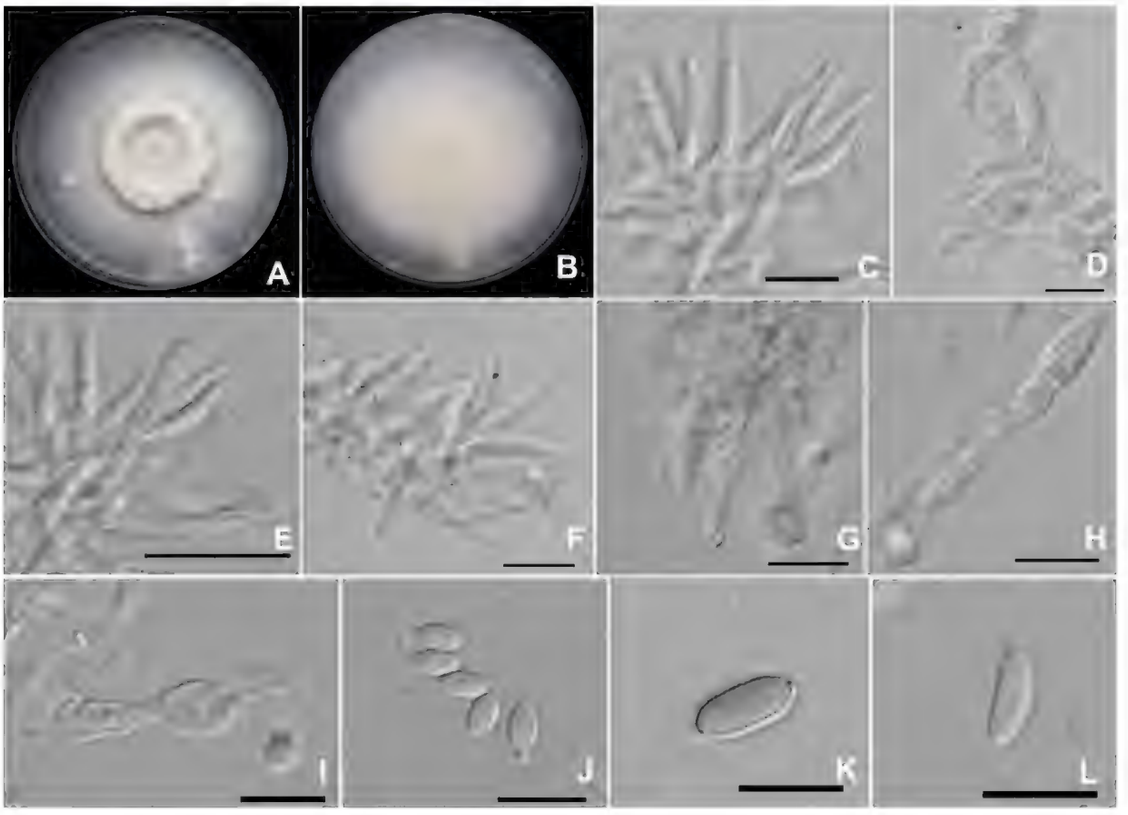

FIG. 2. *Neochrosporium camelliae-sinensis* (holotype, HHAUF220225). A,.B. Colony; C–I. Conidiophores and conidiogenous cells; J–L. Conidia. Scale bars = 20 μm.

COLONY on PDA at 25°C for 7 days reached to 6 cm in diameter, white, velvet or powdery, middle mycelium fluffy, the peripheral mycelium was longer fluffy. MYCELIUM mostly superficial, hyphae smooth, septate, hyaline, 1.0–3.5 μm. CONIDIOPHORES micronematous, mononematous, smooth, hyaline to pale brown, 0–1–septate, 3–5 phialides clustered together, usually branched, 15.0–25.5 × 1.5–4.5 μm. CONIDIOGENOUS CELLS monoblastic, discrete, subcylindrical to doliiform, branches, smooth, hyaline. CONIDIA 0–septate, round or long oval, smooth, thin–walled, hyaline, 5–10 × 2.5–3 μm. Telomorph unkown.

Comments — In the phylogeny, the *Neochrosporium* strain clustered with *Ochrocladosporium* strains (CBS146.33 and CBS 103.81) with 99% bootstrap. Molecular data for *Neochrosporium* and *Ochrocladosporium* are similar, 98% identity between *N. camelliae-sinensis* and *O. elatum*, and they clustered nearest in the phylogenetic tree. *Ochrocladosporium frigidarii* was isolated from cooled room in Germany (Crous & al. 2007), and has 99.5% identity with *O. elatum* in ITS and LSU .

In morphology, *Neochrosporium* can be clearly discriminated from *Ochrocladosporium*. *Ochrocladosporium* colonies are composed of branched and septate mycelium, which produce two kinds of conidia, with the commonly occurring ramoconidia (Crous & al. 2007). The conidia are arranged in chains. But in *Neochrosporium camelliae-sinensis*, colony is white, and ramoconidia absent and the conidia were formed on phialide. Although *Neochrosporium* and *Ochrocladosporium* are very close in phylogeny, but both of ITS and LSU are conserved in fungi (Schoch & al. 2012). The morphology and conidial forms of *Neochrosporium* and *Ochrocladosporium* are relatively different; and as these characters can be effective for diffentiating hyphomycete genera, *Neochrosporium* is supported as a new monotypic genus.

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Literature cited

Crous PW, Braun U, Schubert K, Groenewald JZ. 2007. Delimiting *Cladosporium* from morphologically similar genera. Studies in Mycology 58: 33–56. https://doi.org/10.3114/sim.2007.58.02

Doyle J. 1991. DNA protocols for plants. 283–293, in: GM Hewitt & al. (eds). Molecular Techniques in Taxonomy. NATO ASI Series, vol 57. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-83962-7_18

Fernández FA, Lutzoni FM, Huhndorf SM. 1999. Teleomorph-anamorph connections: the new pyrenomycetous genus *Carpoligna* and its *Pleurothecium* anamorph. Mycologia 91(2): 251–262. https://doi.org/10.1080/00275514.1999.12061015

Fu JM, Aptroot A, Wang ZL, Zhang LL. 2019. Four *Pyrenula* species new to China. Mycotaxon 134(1): 155–160. https://doi.org/10.5248/134.155

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Liu J, Liu CS, Wei SL, Zhang J, Zheng J. 2011. Analysis of rDNA-ITS sequences and similarity of *Radix paeoniae*. Journal of Chinese Medicinal Materials 34(10): 1517–1521.

Lu DS, Wang JP, Wu XQ, Ye JR. 2007 The Species and of Fungi in Tea Trees. Journal of Henan Agricultural Sciences, 2007(10):54-56,80. DOI:10.3969/j.issn.1004-3268.2007.10.016.

Pereira MLS, Carvalho JLVR, Lima JMS, Barbier E, Bernard E, Bezerra JDP, Souza-Motta CM. 2022. Richness of *Cladosporium* in a tropical bat cave with the description of two new species. Mycological Progress 21(1): 345–357. https://doi.org/10.1007/s11557-021-01760-2

Ronquist F, Huelsenbeck J. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. https:// doi.org/10.1093/bioinformatics/btg180

Sayers EW, O'Sullivan C, Karsch-Mizrachi I. 2022. Using GenBank and SRA. Methods in Molecular Biology 2443: 1–25. https://doi.org/10.1007/978-1-0716-2067-0_1

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences of the United States of America 109(16): 6241-6246. https://doi.org/10.1073/pnas.1117018109

Singh R, Kamal. 2012. Two new species of *Corynespora* from northeastern Uttar Pradesh, India. Mycotaxon 118(1): 123–129. https://doi.org/10.5248/118.123

Stielow JB, Lévesque CA, Seifert KA, Meyer W, Irinyi L, Smits D, Renfurm R, Verkley GJM, Groenewald M, Chaduli D & al. 2015. One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. Persoonia 35(1): 242–263. https://doi.org/10.3767/003158515X689135

White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis & al. (eds). PCR protocols: a guide to methods and applications. Academic Press, New York. https://doi.org/10.1016/b978-0-12-372180-8.50042-1